Evidence for the renal paratubular transport of glutathione

Barbara B. Rankin and Norman P. Curthoys

Department of Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA

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1. INTRODUCTION

The catabolism of glutathione in various non-renal tissues occurs through an interorgan process [1,2]. For example, the turnover of glutathione within rat liver is initiated by the unidirectional release of glutathione to the blood plasma [3]. The released glutathione is carried to the kidney where it is nearly quantitatively extracted and degraded to its constituent amino acids [4,5]. The glutathionemia and the pronounced glutathionuria observed in a patient who lacks detectable γ-glutamyltranspeptidase [6] and in mice treated with inhibitors of γ-glutamyltranspeptidase [7,8] indicate that this enzyme catalyzes the initial reaction in glutathione catabolism. The transpeptidase is an amphipathic membrane glycoprotein [9] that is found to the greatest extent in the kidney. Within this tissue, the enzyme is primarily associated with the brush border membrane of the proximal tubule cells [10], where it is asymmetrically orientated on the lumenal surface [11].

Glutathione contained within the kidney also turns over rapidly [12]. The initial step in this process is the translocation of glutathione from the epithelial cells to the tubular lumen [7,8]. Here, the secreted renal glutathione is degraded and the resulting amino acids presumably enter the same pool as produced by the degradation of non-renal glutathione. Reabsorption of the amino acids and their redistribution for protein synthesis or the resynthesis of glutathione completes the interorgan metabolism of glutathione.

The percent of plasma glutathione extracted by the kidney greatly exceeds the percent filtered by the glomeruli even when the arterial glutathione concentration is increased to a value 200-fold greater than normal [5]. Therefore, the kidney may contain a mechanism for the transport of glutathione across the basolateral plasma membrane. However, small amounts of γ-glutamyltranspeptidase may be associated with the glomeruli [13], with the renal microvasculature [14], or with the basolateral membrane of the proximal tubule [15]. Thus, the apparent extraction of glutathione by the kidney may be due to its catabolism within the post-glomerular paratubular space. In order to resolve these two possibilities, we have used the procedures in [16] to study the renal paratubular handling of glutathione.

2. MATERIALS AND METHODS

White male rats (150–250 g) were obtained from Zivic Miller and were maintained on Purina Rat Chow. All of the radioisotopes used in these experiments were purchased from New England Nuclear. The AT-125 (L-(α*S*,5*S*)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) was obtained from Dr Ruth Davis of the National Cancer Institute. γ-Glutamyltranspeptidase activity [17] and glutathione concentration [18] were determined as described.

To study the paratubular handling of glutathione, rats were initially anesthetized with sodium pentothal and injected with heparin. Cannulae were inserted into the abdominal aorta and the inferior vena cava below the left kidney. Ligatures were then tied around the coeliac, mesenteric and right renal arteries and around the vena cava

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just above the left renal vein. A 50 μl sample containing [^{14}C]inulin and [*glycine*-^{3}H]glutathione was injected into the aorta and the entire effluent from the vena cava was collected dropwise into separate scintillation vials. The drops of blood were solubilized with NCS tissue solubilizer, cleared with H_2O_2 and diluted with ACS scintillation solution. The ^{3}H and ^{14}C radioactivities were determined for each sample and the ratio of counts was normalized by dividing by the ^{3}H/^{14}C ratio determined for the sample initially injected. A value of < 1 for the normalized ratio indicates that the substance being studied is transported out of the post-glomerular paratubular space [16].

3. RESULTS AND DISCUSSION

The validity of the single-pass perfusion technique for studying paratubular transport was initially established by arterial infusion of a sample containing *p*-amino[^{14}C]hippurate and [^{3}H]inulin (fig.1). The [^{3}H]inulin was recovered in the venous effluent as a single peak of radioactivity. However, a much smaller proportion of the injected *p*-amino[^{14}C]hippurate was recovered. The normalized ^{14}C/^{3}H ratio for the collected samples varied from 0.1–0.2 indicating that >80% of the *p*-aminohippurate contained in the paratubular blood was extracted across the basolateral plasma membrane. This result is consistent with experiments characterizing the paratubular uptake of *p*-aminohippurate [19].

The paratubular extraction of glutathione was then characterized by infusion of a sample containing [*glycine*-^{3}H]glutathione and [^{14}C]inulin (fig.2). The recovery of ^{3}H radioactivity was significantly decreased compared to the recovery of [^{14}C]inulin. The initial fractions collected from the venous effluent exhibited a ^{3}H/^{14}C ratio of only 0.25. However, in later fractions, the radioactivity ratio increased steadily and it reached a value of 0.9 in the last sample collected. The observed pattern indicates that glutathione may be efficiently transported across the basolateral membrane. The increase in the ^{3}H/^{14}C ratio observed in the later fractions could indicate that the extracted glutathione is metabolized and the resulting products are returned to the paratabular circulation.

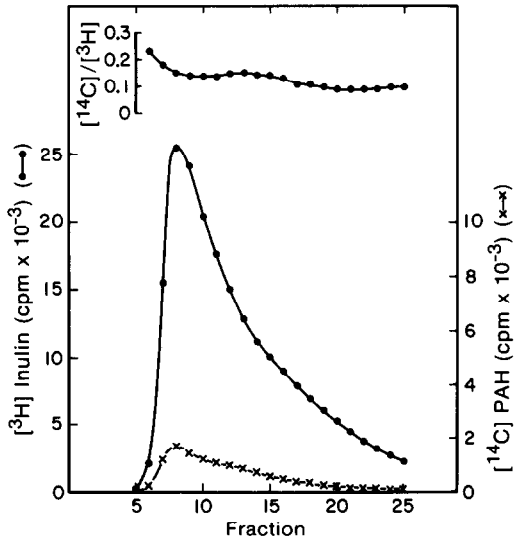

Fig.1. Renal paratubular extraction of *p*-aminohippurate. A 50 μl-sample containing *p*-amino[^{14}C]hippurate (PAH) and [^{3}H]inulin was injected into the abdominal aorta and the total effluent from the renal vein was collected dropwise. The ^{14}C/^{3}H ratio for each fraction was normalized by dividing by the ^{14}C/^{3}H ratio of radioactivity contained in the initial sample.

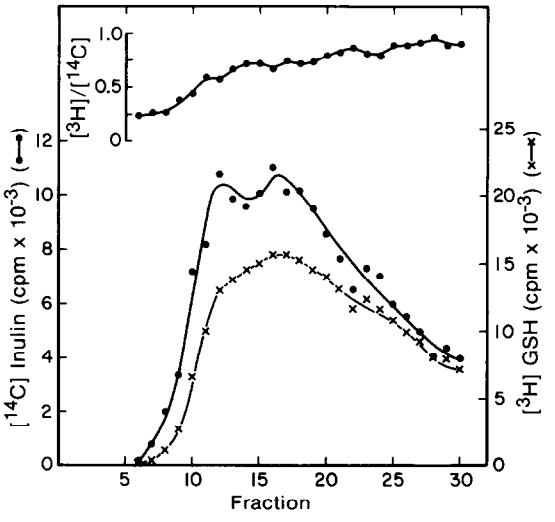

Fig.2. Renal paratubular extraction of glutathione. A 50 μl sample containing [*glycine*-^{3}H]glutathione (GSH) and [^{14}C]inulin was injected into the abdominal aorta of an untreated rat. The ^{3}H/^{14}C ratio for each fraction was normalized by dividing by the ^{3}H/^{14}C ratio of radioactivity contained in the initial sample.

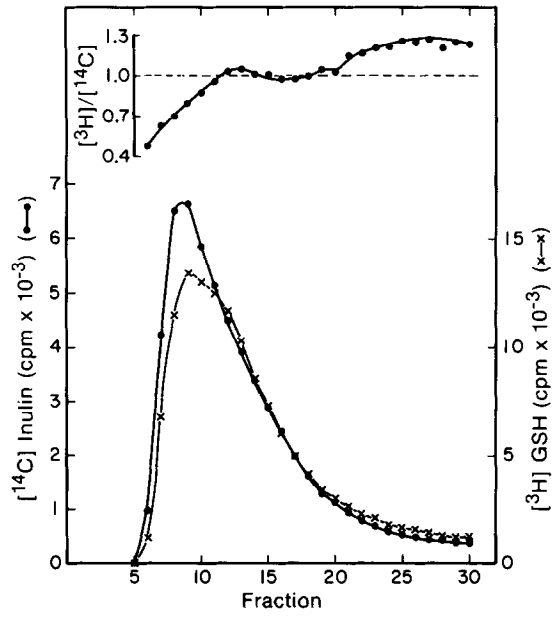

Fig.3. Renal paratubular extraction of glutathione in an AT-125-treated rat. The experiment was done as in fig.2 except that the rat received an i.v. injection of 0.05 mmol AT-125/kg body wt 1 h before infusion of labeled glutathione (GSH).

Table 1

Effect of inactivation of γ-glutamyltranspeptidase on the renal handling of glutathione

Parameter	Normal	AT-125 treated[a]
γGT Activity (μmol/min . mg)	1.23 ± 0.08	0.026 ± 0.006
Urinary [GSH] (μM)	2.8 ± 0.5	7400 ± 1300
Arterial [GSH] (μM)	12.8 ± 0.7	30.4 ± 2.9
Venous [GSH] (μM)	2.6 ± 0.7	8.3 ± 1.8
Renal extraction	80%	73%

[a] γ-glutamyltranspeptidase (γGT) activity and plasma levels of glutathione (GSH) were determined 1 h after an i.v. injection of 0.05 mmol AT-125/kg body wt, whereas urinary glutathione was determined on samples collected for 4 h

However, the observed results are also consistent with the catabolism of glutathione within the paratubular plasma and the partial extraction of the resulting amino acids.

To distinguish between the 2 alternative possibilities, rats were pretreated with AT-125. This compound is a potent affinity label, which causes the in vivo inactivation of γ-glutamyltranspeptidase [20]. Renal γ-glutamyltranspeptidase is inhibited 98% within 1 h after injecting AT-125 (table 1). This treatment has no effect on the plasma concentration or the urinary excretion of amino acids. However, it does result in a 3000-fold increase in the urinary concentration of glutathione indicting that a physiologically significant proportion of the γ-glutamyltranspeptidase activity is inhibited. AT-125 treatment also produces a significant increase (2.5-fold) in the arterial concentration of glutathione. A comparison of arterial and renal venous concentrations indicates that the inhibition of γ-glutamyltranspeptidase has little effect on the ability of the kidney to extract plasma glutathione. The 73% extraction observed in AT-125-treated rats greatly exceeds the proportion of plasma glutathione removed by glomerular filtration. These results are in contrast to those reported in [21], which used a 50-fold greater dose of AT-125 and observed that renal extraction of glutathione was decreased to 39% while the γ-glutamyltranspeptidase activity was inhibited only 90–95%. When used in high concentrations AT-125 becomes a non-specific alkylating agent [22], that may inhibit the paratubular transport system for glutathione.

The paratubular extraction of glutathione was then characterized in AT-125-treated rats. The data shown in fig.3 indicate that compared to the recovery of [^{14}C]inulin a significant extraction of [^{3}H]glutathione was still observed. The normalized $^{3}H/^{14}C$ ratio observed in the initial fraction was 0.4. However, this ratio increased in later fractions and it reached a value slightly greater than 1. Since 98% of the γ-glutamyltranspeptidase activity was inhibited in this experiment, it is unlikely that the labeled glutathione released to the paratubular circulation is catabolized. The observation that AT-125 treatment does not block renal extraction or paratubular uptake of glutathione provides strong evidence for the existence of a transport system for

glutathione within the basolateral membrane. It is now important to characterize the specificity of this transport system to determine if it can account for the ability of the kidney to extract various γ-glutamyl compounds from the blood [23,24].

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